Efficient generation of genome-modified mice using Campylobacter jejuni-derived CRISPR/Cas

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Supplementary Information

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Supplementary Figure 1. Sequences of Cj-Cas9 and gRNAs.

>DNA sequence of Cj-Cas9 vector (from T3 promoter to SphI site)

GAAATTAACCCTCACTAAAGGGAACAAAAGCTGGAGCTCCACCGCGGTGGCGGCCGCGGGAATTCGA
TTCCACCATGGACTATAAGGACGACGACGATAAGATAATGGCCCCTAAGAAGAAAAGAAAGGTGGG
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CGAATTGAAGGACTGCGGAGTTAGGATCTTCACTAAAGTAGAGAACCCTAAGACTGGTGAATCCCTG
GCACTGCCCAGAAGACTGGCTAGGAGCGCACGGAAGCGACTCGCTCGCAGAAAGGCAAGACTTAAT
CACCTTAAACACCTTATTGCAAACGAGTTCAAGCTCAATTACGAAGATTATCAGTCTTTTGACGAAAG
CCTTGCCAAGGCCTACAAAGGTAGTCTCATCAGCCCCTACGAACTCAGGTTCAGGGCACTGAATGAA
CTCCTGTCCAAGCAGGATTTCGCAAGAGTCATACTTCACATAGCTAAACGGAGGGGCTATGATGACA
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TTGCTAACTACCAATCAGTAGGAGAATACCTGTATAAGGAATATTTTCAGAAGTTTAAGGAAAACTCC
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AGGATGAGCTGAAACTTATATTCAAAAAGCAAAGGGAGTTCGGATTCTCCTTTAGCAAAAAATTTGA
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GCTCTTTCTTTACCGATGAGAAACGCGTACCTAAGAATAGTCCACTCGCTTTTATGTTTGTCGCTTTGA
CACGAATTATAAACCTGCTGAATAACCTTAAAAATACAGAGGGCATCCTGTACACAAAGGATGATCT
CAATGCTCTGTTGAATGAAGTTCTCAAGAACGGTACTCTGACTTATAAACAAACAAAGAAGCTGCTC
GGTCTGTCAGACGATTATGAGTTTAAGGGCGAAAAGGGGACCTACTTTATCGAGTTTAAGAAGTATA
AAGAATTCATCAAAGCCCTGGGAGAGCATAACTTGTCACAAGATGATCTTAATGAGATTGCAAAAGA
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ACATCTACCCATATTCAAGATCTTTCGACGACTCCTACATGAATAAAGTCCTCGTTTTTACCAAACAG
AATCAAGAGAAGCTCAACCAGACCCCCTTTGAGGCCTTCGGCAACGACAGTGCAAAGTGGCAAAAAA
TAGAAGTCCTCGCTAAAAATCTCCCCACAAAAAAACAAAAGAGGATATTGGACAAGAACTATAAAG
ATAAGGAGCAAAAAAACTTTAAAGACAGGAATCTTAACGACACTAGATACATCGCTAGACTGGTATT
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AGAGGGTGTTCTTAAAGCACTCGAGCTTGGAAAGATCCGGAAGGTGAACGGGAAGATAGTCAAAAA
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GAATGCTAACGAGAAGGAGGTGATTGCAAAGAGCATTGGCATTCAAAACCTGAAAGTGTTTGAAAAA
TACATCGTTTCAGCACTGGGAGAAGTAACAAAGGCTGAATTTAGGCAAAGAGAGGACTTCAAGAAAG
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TAAACGTTATCTTTTTAAATTGAATGTGCACAAATAAAAGTTTGGAAAAGAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAACCCTCGAGGTAGCATGC

>Amino acid sequence of Cj-Cas9
MDYKDDDDKIMAPKKKRKVGSMARILAFDIGISSIGWAFSENDELKDCGVRIFTKVENPKTGESLALPRRL
ARSARKRLARRKARLNHLKHLIANEFKLNYEDYQSFDESLAKAYKGSLISPYELRFRALNELLSKQDFARV
ILHIAKRRGYDDIKNSDDKEKGAILKAIKQNEEKLANYQSVGEYLYKEYFQKFKENSKEFTNVRNKKESY
ERCIAQSFLKDELKLIFKKQREFGFSFSKKFEEEVLSVAFYKRALKDFSHLVGNCSFFTDEKRVPKNSPLAF
MFVALTRIINLLNNLKNTEGILYTKDDLNALLNEVLKNGTLTYKQTKKLLGLSDDYEFKGEKGTYFIEFKK
YKEFIKALGEHNLSQDDLNEIAKDITLIKDEIKLKKALAKYDLNQNQIDSLSKLEFKDHLNISFKALKLITPL
MLEGKKYDEACNELNLKVAINEDKKDFLPAFNETYYKDEVTNPVVLRAIKEYRKVLNALLKKYGKVHKI
NIELAREVGKNHSQRAKIEKEQNENYKAKKDAELECEKLGLKINSKNILKLRLFKEQKEFCAYSGEKIKISD
LQDEKMLEIDHIYPYSRSFDDSYMNKVLVFTKQNQEKLNQTPFEAFGNDSAKWQKIEVLAKNLPTKKQK
RILDKNYKDKEQKNFKDRNLNDTRYIARLVLNYTKDYLDFLPLSDDENTKLNDTQKGSKVHVEAKSGML
TSALRHTWGFSAKDRNNHLHHAIDAVIIAYANNSIVKAFSDFKKEQESNSAELYAKKISELDYKNKRKFFE
PFSGFRQKVLDKIDEIFVSKPERKKPSGALHEETFRKEEEFYQSYGGKEGVLKALELGKIRKVNGKIVKNG
DMFRVDIFKHKKTNKFYGVPIYTMDFALKVLPNKAVARSKKGEIKDWILMDENYEFCFSLYKDSLILIQTK
DMQEPEFVYYNAFTSSTVSLIVSKHDNKFETLSKNQKILFKNANEKEVIAKSIGIQNLKVFEKYIVSALGEV
TKAEFRQREDFKKGSMAPKKKRKV

>gRNA template vector (from T3 promoter to DraI site)
GAAATTAACCCTCACTAAAGGAGAGACGATCGTCTCTGTTTTAGTCCCTGAAAAGGGACTAAAATAA
AGAGTTTGCGGGACTCTGCGGGGTTACAATCCCCTAAAACCGCTTTTAAA

>gRNA T1 (from T3 promoter to DraI site)
GAAATTAACCCTCACTAAAGGAGCACTGGCAGGTCCTATTAGTTTTAGTCCCTGAAAAGGGACTAA
AATAAAGAGTTTGCGGGACTCTGCGGGGTTACAATCCCCTAAAACCGCTTTTAAA

>gRNA T2 (from T3 promoter to DraI site)
GAAATTAACCCTCACTAAAGGGGTTGCTGGAAAAGAAGTCGTTTTAGTCCCTGAAAAGGGACTAAA
ATAAAGAGTTTGCGGGACTCTGCGGGGTTACAATCCCCTAAAACCGCTTTTAAA

>gRNA T3 (from T3 promoter to DraI site)
GAAATTAACCCTCACTAAAGGAGATCTGGAAACTCCACAGAAGTTTTAGTCCCTGAAAAGGGACTA
AAATAAAGAGTTTGCGGGACTCTGCGGGGTTACAATCCCCTAAAACCGCTTTTAAA

>gRNA T4 (from T3 promoter to DraI site)
GAAATTAACCCTCACTAAAGGAGCATGAAAATGTGGCTGCGTTTTAGTCCCTGAAAAGGGACTAAA
ATAAAGAGTTTGCGGGACTCTGCGGGGTTACAATCCCCTAAAACCGCTTTTAAA

>gRNA T5 (from T3 promoter to DraI site)
GAAATTAACCCTCACTAAAGGAGGGCCAGGACTCACGGTCGTTTTAGTCCCTGAAAAGGGACTAAA
ATAAAGAGTTTGCGGGACTCTGCGGGGTTACAATCCCCTAAAACCGCTTTTAAA

>gRNA T6 (from T3 promoter to DraI site)
GAAATTAACCCTCACTAAAGGAAAGCTGGCCGCAGGGACTCCGTTTTAGTCCCTGAAAAGGGACTA
AAATAAAGAGTTTGCGGGACTCTGCGGGGTTACAATCCCCTAAAACCGCTTTTAAA

>gRNA T7 (from T3 promoter to DraI site)
GAAATTAACCCTCACTAAAGGGGTGGATGACCGTGAGTGTTTTAGTCCCTGAAAAGGGACTAAAAT
AAAGAGTTTGCGGGACTCTGCGGGGTTACAATCCCCTAAAACCGCTTTTAAA

>gRNA T8 (from T3 promoter to DraI site)
GAAATTAACCCTCACTAAAGGCACTGGCAGGTCCTATTATAGTTTTAGTCCCTGAAAAGGGACTAAA
ATAAAGAGTTTGCGGGACTCTGCGGGGTTACAATCCCCTAAAACCGCTTTTAAA

>gRNA T9 (from T3 promoter to DraI site)
GAAATTAACCCTCACTAAAGGAAGGCAATACAAAACAGCCGTTTTAGTCCCTGAAAAGGGACTAAA
ATAAAGAGTTTGCGGGACTCTGCGGGGTTACAATCCCCTAAAACCGCTTTTAAA

>gRNA T10 (from T3 promoter to DraI site)
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ATAAAGAGTTTGCGGGACTCTGCGGGGTTACAATCCCCTAAAACCGCTTTTAAA

>gRNA T11 (from T3 promoter to DraI site)
GAAATTAACCCTCACTAAAGGCCTGCCAGGATATCCTTCTGTCGTTTTAGTCCCTGAAAAGGGACTA
AAATAAAGAGTTTGCGGGACTCTGCGGGGTTACAATCCCCTAAAACCGCTTTTAAA

>gRNA T12 (from T3 promoter to DraI site)
GAAATTAACCCTCACTAAAGGAAGGGGAACTGAGGTCCAGAGTTTTAGTCCCTGAAAAGGGACTAA
AATAAAGAGTTTGCGGGACTCTGCGGGGTTACAATCCCCTAAAACCGCTTTTAAA

>gRNA T13 (from T3 promoter to DraI site)
GAAATTAACCCTCACTAAAGGAAGTTTGGATTTGGGGGCCCAGTTTTAGTCCCTGAAAAGGGACTA
AAATAAAGAGTTTGCGGGACTCTGCGGGGTTACAATCCCCTAAAACCGCTTTTAAA

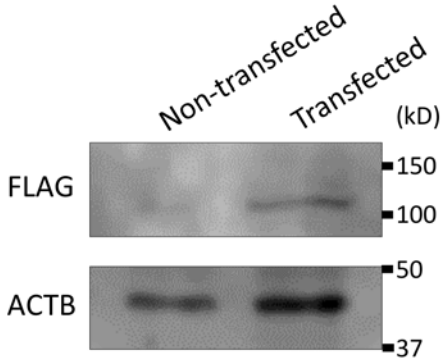
>gRNA R1 (from T3 promoter to DraI site)
GAAATTAACCCTCACTAAAGGAGAACTCCCAGAAAGGTATGTTTTAGTCCCTGAAAAGGGACTAAA
ATAAAGAGTTTGCGGGACTCTGCGGGGTTACAATCCCCTAAAACCGCTTTTAAA

>gRNA R2 (from T3 promoter to DraI site)
GAAATTAACCCTCACTAAAGGAGTGGAGTAGGCGGGGAGAAGTTTTAGTCCCTGAAAAGGGACTAA
AATAAAGAGTTTGCGGGACTCTGCGGGGTTACAATCCCCTAAAACCGCTTTTAAA

>gRNA R3 (from T3 promoter to DraI site)
GAAATTAACCCTCACTAAAGGTGGGAAGTCTTGTCCCTCCAAGTTTTAGTCCCTGAAAAGGGACTAA
AATAAAGAGTTTGCGGGACTCTGCGGGGTTACAATCCCCTAAAACCGCTTTTAAA

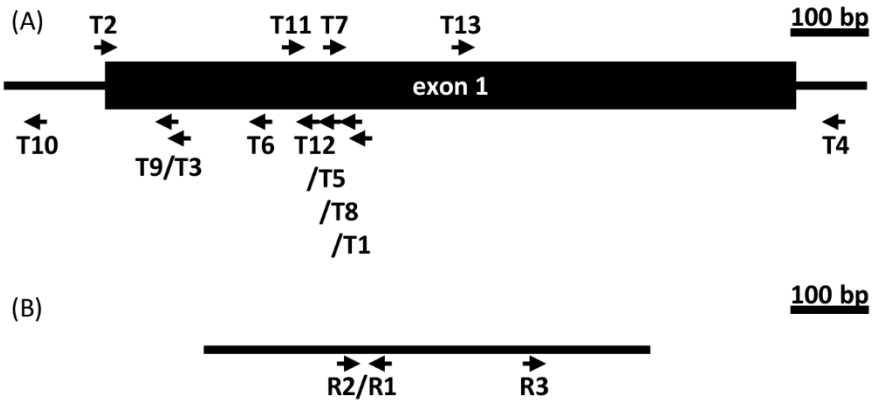
>gRNA #1 for Chk2 (from T3 promoter to DraI site)
GAAATTAACCCTCACTAAAGGCACATTCAAAGCTCACAACACAGGTTTTAGTCCCTGAAAAGGGACT
AAAATAAAGAGTTTGCGGGACTCTGCGGGGTTACAATCCCCTAAAACCGCTTTTAAA

>gRNA #2 for Chk2 (from T3 promoter to DraI site)
GAAATTAACCCTCACTAAAGGCATTTCTTTCACATTCAAAGCTGTTTTAGTCCCTGAAAAGGGACTAA
AATAAAGAGTTTGCGGGACTCTGCGGGGTTACAATCCCCTAAAACCGCTTTTAAA



Supplementary Figure 2. Confirmation of Cj-Cas9 expression by immunoblotting.

A band with expectative molecular weight (about 118 kD) of Cas9 protein was observed in HEK293 cells. Beta-actin (ACTB) was shown as loading control.



(C)

gRNA	Target sequence	PAM	No. of blastocyst / 2-cell embryo (%)
T1	GAGCACTGGCAGGTCCTATTA	TAAAACAC	29/32 (90.6)
T2	GGGGTTGCTGGAAAAGAAGTC	TGTGACAC	24/26 (92.3)
T3	GAGATCTGGAAACTCCACAGAA	GGCAATAC	20/22 (90.9)
T4	GAGCATGAAAATGTGGCTGC	TGAAGTAC	24/26 (92.3)
T5	GAGGGCCAGGACTCACGGTC	ATCCACCC	19/20 (95)
T6	GAAAGCTGGCCGCAGGGACTCC	CATCACCC	18/18 (100)
T7	GGGGTGGATGACCGTGAGT	CCTGGCCC	30/30 (100)
T8	CACTGGCAGGTCCTATTATA	AAACACAG	16/16 (100)
T9	GAAGGCAATACAAAACAGCC	AAGAACAT	18/20 (90)
T10	CATCAAATACTCTCAGCCTG	TTTTACAC	23/25 (92)
T11	CCTGCCAGGATATCCTTCTGTC	CAGTGCAC	17/18 (94.4)
T12	AAGGGGAACTGAGGTCCAGA	TGGTGCAC	16/16 (100)
T13	AAGTTTGGATTTGGGGGCCCA	AATTGTAC	20/21 (95.2)
R1	GAGAACTCCCAGAAAGGTAT	TGCAACAC	24/26 (92.3)
R2	AGTGGAGTAGGCGGGGAGAA	GGCCGCAC	21/23 (91.3)
R3	GTGGGAAGTCTTGTCCCTCCAA	TTTTACAC	18/18 (100)

Supplementary Figure 3. Schematics of target loci of each gRNAs.

(A) The gRNAs designed in Tyrosinase locus (Chr7: 87492422 to 87493521 in GRCm38.p4). (B) The gRNAs designed in Rosa26 locus (Chr6: 113075796 to 113076364 in GRCm38.p4). Each arrow indicates the target of gRNAs shown in Table 1 and Supplementary Figure 1. The direction of arrow means the 5' to 3' of gRNA recognition sequence. (C) Target sequences of each gRNAs and the developmental competencies of the microinjected embryos.

Supplementary Table 1. Primer sets for synthesis of Cj-gRNA template vectors and ssODN for Chk2-Flag knock-in.

Target	Sequences
Fw primer for platform gRNA	5’-GAAATTAACCCTCACTAAAGGAGAGACGATCGTCTCTGTTTTAGTCCCTGAAAAGGGACTAAAATAAAGA-3’
Rv primer for platform gRNA	5’-TTTAAAAGCGGTTTTAGGGGATTGTAACCCCGCAGAGTCCCGCAAACTCTTTATTTTAGTCCCTTTTCAG-3’
ssODN for Chk2-Flag	5’-GAACTGGAGGTGGAGGGTATGCCGAGCACAAAACGCCTGTCTGTGTGTGGGGCTGTGTTGGGATCCGATTACAAGGA TGACGACGATAAGATCTGAGCTTTGAATGTGAAAGAAATGTGCTTCCTTCCACGCTCCAATTGTCTTCTCCATCTTG-3’

Supplementary Table 2. Primer sets for genomic PCR.

Target locus		Sequences	
Tyrosinase target	Forward 1	5'-	TTATGCATTGAAGCAGTTCACC
	Reverse 1	5'-	TTCCAGATACCATAGGAGGTGC
	Forward 2	5'-	TTTGACAGTGGTGGAAACTGTCC
	Reverse 2	5'-	AGCAATGTTACTTCGCAGCAGAGC
Tyrosinase off-target-1	Forward	5'-	GGAGACAAAGTGCAGAGAAGAG
	Reverse	5'-	GGGGGTCTGGTTGATAATGTTG
Tyrosinase off-target-2	Forward	5'-	GACATCAGCAACATTTTCCAAG
	Reverse	5'-	CTTTGCAAGGTTCAACAGAGTG
Tyrosinase off-target-3	Forward	5'-	TATTCCCCTACACTGGGTCATC
	Reverse	5'-	TGGCTAATGAGGGAGATAAAACCAG
Rosa26 locus	Forward	5'-	ACGTTTCCGACTTGAGTTGC
	Reverse	5'-	ATACTCCGAGGCGGATCAC
Chk2	Forward	5'-	AGGTGTGTGAGGAGGATTCG
	Reverse	5'-	GTGCTGGGATTAAAGGAGTGG

Supplementary Table 3. On-target and off-target loci of *Tyrosinase*.

Target	Locus	Target sequences	Mutated/Total pups
Tyrosinase target	chr7: 87493021-87493158	GAGCACTGGCAGGTCCTATTATAAAACAC	20/20
Off-target-1	chr3: 61987856-61987993	AGGCTCTGGCAGGGCCTCTTAGAAGACAC	0/20
Off-target-2	chr8: 97686169-97686306	AGGTTCTGGCAGGGCCTCTTAGGGGACAC	0/20
Off-target-3	chr6: 8891204-8891341	GACACCTGGCAGGTCCATTTAGAACACAC	0/20